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Developmental toxicity of N-methyl-2-pyrrolidone administered orally to rats

A.M. Saillenfait*, F. Gallissot, I. Langonné, J.P. Sabaté

Institut National de Recherche et de Sécurité, Avenue de Bourgogne, BP No. 27, 54501 Vandoeuvre, France

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Abstract

The developmental toxicity of N-methyl-2-pyrrolidone (NMP) was studied in Sprague–Dawley rats after oral administration. Pregnant rats were given NMP at doses of 0 (distilled water), 125, 250, 500, and 750 mg/kg/day, by gavage, on gestational days (GD) 6 through 20. Significant decreases in maternal body weight gain and food consumption during treatment, and a reduction in absolute weight gain were observed at 500 and 750 mg/kg. The incidence of resorptions per litter was significantly higher than control at 500 mg/kg, and rose to 91% at 750 mg/kg. Examination of the foetuses revealed treatment-related malformations, includingimperforate anus and absence of tail, anasarca, and malformations of the great vessels and of the cervical arches. The incidence of malformed foetuses per litter, and of litters with malformed foetuses was significantly increased at 500 and 750 mg/kg. At 250 mg/kg, one foetus showed malformations similar to those recorded at higher dosages. There was a dose-related decrease in foetal body weights (male, female, and total) that reached statistical significance at 250 mg/kg. A significant increase in incomplete ossification of skull bones and of sternebrae was also present at 500 and 750 mg/kg. In summary, the no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity was 250 and 125 mg/kg/day, respectively. Thus, oral administration of NMP produced developmental toxicity below maternally toxic levels.

Keywords: Developmental toxicity; Rat; N-Methyl-2-pyrrolidone

1. Introduction

N-Methyl-2-pyrrolidone (NMP, CAS no. 872–50–4) is a common polar solvent. It is used widely in a variety of chemical reactions, and in the manufacture of numerous chemical intermediates and products such as plastics, surface coatings and pesticides. It is also used as a medium for polymerisation, as a solvent for finished polymers, and for extraction in the petrochemical industry. Another important application of NMP is paints and polymers strippingand cleaning(e.g in the electronic industry or for removal of graffiti, Health and Safety Executive, 1997). US production reached 65–70 million lbs in 1991 (Solomon et al., 1996). NMP may enter the environment duringproduction and use. If released into water or soil, screeningstudies indicate that NMP will rapidly biodegrade under aerobic conditions. It is not expected to bioconcentrate significantly in fish and aquatic organisms (Trochimowicz et al., 2001).

The developmental toxic potential of NMP has been investigated previously in rats following dermal and inhalation exposure. Time-pregnant rats were exposed by whole body inhalation to 0, 100 or 360 mg/m^3 aerosolised NMP, for 6 h per day, from gestational day (GD) 6–15 (Lee et al., 1987). Maternal toxicity limited to sporadic lethargy and irregular respiration was observed in several dams duringthe first 3 days of treatment. No evidence of developmental toxicity was found. As a part of a two-generation reproduction study, male and female rats were exposed whole body to 0 or 116 ppm NMP vapours (478 mg/m^3), 6 h per day, 7 days a week, for at least 14 weeks (Solomon et al., 1995). In the case of females, NMP exposure was maintained throughout gestation until GD 20. Exposed males were mated with exposed females. Pregnant animals were euthanized on GD 21 and the foetuses were

Abbreviations: GD, gestational day; LOAEL, lowest-observable-adverse-effect level; NMP, N-methyl-2-pyrrolidone; NOAEL, no-observed-adverse-effect level

* Correspondingauthor. Tel.: +33-3835 02039; fax: +33-3835 09846.

E-mail address: anne-marie.saillenfait@inrs.fr (A.M. Saillenfait).

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examined for developmental toxic effects. NMP caused marginal maternal/parental toxicity (i.e. transient decrease in response to noise) and the only sign of toxicity detected in offspringwas a reduction of the foetal body weight. A decrease in pup weight was also detected amongnewborns in the reproductive phase of this study in which the dams were allowed to deliver. Hass et al. (1995) exposed pregnant rats to 165 ppm NMP (680 mg/m^3), 6 h per day, on GD 4–20. The authors claimed that this vapour concentration of NMP was the highest possible that could be attained under their experimental conditions. Developmental toxicity, indicated by increased pre-implantation loss, delayed ossification of the skeleton and decreased foetal body weight, was observed in the absence of overt signs of maternal toxicity. NMP was administered cutaneously to pregnant rats at doses levels of 0, 75, 237 or 750 mg/kg on GD 6–15 (Becci et al., 1982). Application of 750 mg/kg resulted in depressed maternal body weight gain, increased incidence of resorptions, and reduced number of live foetuses and mean foetal body weight. In addition, the incidence of several skeletal defects was increased, includingincomplete ossification of sternebrae or vertebrae, fused/split or extra ribs, fused atlas and occipital bones, and reduced ossification of skull bones. There were no signs of maternal or embryo/foetal toxicity at lower doses.

It has been reported that NMP can be transferred to the foetus after inhalation by pregnant rats at term (Ravn-Jonsen et al., 1992).

Despite its widespread uses, there is no information in the published literature regarding the developmental toxic effects of NMP after oral administration. Therefore, the study reported here was conducted to assess the developmental toxic effects of NMP administered by gavage to Sprague–Dawley rats throughout the embryonic and foetal period. The results of this investigation, designed in accordance with the current regulatory guidelines, provide additional information relevant to the safety evaluation of NMP exposure duringpregnancy.

2. Materials and methods

2.1. Animals

After 1–2 weeks of acclimatization, primiparous female (200–220 g) Sprague–Dawley rats supplied by IFFA CREDO BreedingLaboratories (Saint-Germain-sur-l'Arbresle, France) were housed overnight with adult males from the same strain and supplier. The day sperm was detected in the vaginal smear was considered to be day 0 of gestation (GD). Mated females were singly housed in clear polycarbonate cages with stainless-steel wire lids and corn cob granules as bedding. Food pellets (UAR Alimentation Villemoisson, France) and filtered tap water were available ad lib. Animal rooms were maintained at 21ff 2 ^{fff}C, a relative humidity of 50ff 5%, and a 12-h light/dark photocycle. Mated females were randomly assigned to treatment groups by stratified randomisation so that mean body weights on GD 0 did not differ amongtreatment groups.

2.2. Test chemical and treatment

N-Methyl-2-pyrrolidone (NMP, 5 99.5% pure) was purchased from Merck (Darmstadt, Germany). Dosing solutions were formulated in distilled water as vehicle. Pregnant females were given daily doses of NMP, at approximately the same time each day, by gastric intubation on GD 6–20. The dosingvolume was 5 ml/kg Initial doses were based on GD 6 weight and adjusted every 3 days throughout the treatment period. A control group received the distilled water vehicle under the same conditions.

2.3. Dose selection

Two exploratory dose-range finding studies were conducted to generate data for the selection of dose levels for the definitive study. In the first, pregnant rats (15–16 animals per treatment group) were given doses of 500, 1000 or 1500 mg/kg NMP. No test dams died. Maternal body weight gains were significantly depressed in all NMP-treated groups. Administration of 1000 and 1500 mg/kg resulted in complete early resorptions in all litters. Significantly increased embryolethality (13.8% resorptions vs 4.7% in the control group) and decreased foetal body weight were observed at 500 mg/kg. In addition, four foetuses exhibited external malformations includingimperforate anus and absence of tail (four cases), proboscis and cyclopia (one case). In the second pilot study, pregnant rats (10–12 animals per treatment group) were given doses of 500, 625 or 750 mg/kg NMP. Significant decreases in maternal body weight gain during the treatment period occurred at all dose levels. A dose-dependent increase in the percentage of resorptions per litter and a decrease in the foetal body weight were noted. External foetal observation revealed one foetus with imperforate anus and absence of tail at 500 mg/kg, and three foetuses with anasarca at 625 mg/kg. No visceral and skeletal examinations were conducted in either of these preliminary studies.

Based on the results of these studies, the dose levels selected for the definitive developmental toxicity study were 125, 250, 500 or 750 mg/kg NMP.

2.4. Observations

Group size ranged from 25–27 time-mated rats (21–25 pregnant). All females were observed daily for clinical

signs of toxicity. Food consumption was measured at 3-day intervals startingon GD 6. Maternal body weights were recorded on GD 0, 6, 9, 12, 15, 18 and 21. On GD 21, the females were killed by an intrapulmonary injection of T61 (Hoechst AG, Frankfurt, Germany) and the uterus removed and weighed. Uterine contents were examined to determine the number of implantation sites, resorptions, and dead and live foetuses. The number of corpora lutea in each ovary was recorded. Uteri which had no visible implantation sites were stained with ammonium sulphide (10%) to detect very early resorptions (Salewski, 1964). Live foetuses were weighed, sexed, and examined for external anomalies includingthose of the oral cavity. Half of the live foetuses from each litter were preserved in Bouin's solution and examined for internal soft tissue changes (Wilson, 1965; Barrow and Taylor, 1969). The other half were fixed in ethanol (70%), eviscerated, and then processed for skeletal stainingwith Alizarin Red S for subsequent skeletal examination (Staples and Schnell, 1964).

2.5. Statistical analysis

Whenever possible, the data were presented as mean ± S.D. The number of corpora lutea, implantation sites and live foetuses and various body weights were analyzed by one-way analysis of variance, followed by Dunnett's test if differences were found. The frequency of post-implantation loss, dead foetuses, resorptions and alterations amonglitters was evaluated by usingthe Kruskal–Wallis test followed by the Mann–Whitney test where appropriate. Rates of pregnancy and incidences of foetal alterations per dose were analysed by using Fisher's test. Where applicable, least-squares analysis was carried out. The reported level of statistical significance was $P < 0.05$. The litter was used as the basis for the analysis of foetal variables.

3. Results

All females survived to their scheduled euthanization. There were no clinically significant signs of toxicity attributable to NMP treatment. Excretion of bright yellow-coloured urine was noted amonganimals in all the treated groups. No statistically significant changes were detected in the body weight gain and food consumption at 125 and 250 mg/kg (Table 1). Maternal body weight was significantly decreased from GD 15–21 at 500 mg/kg, and from GD 12–21 at 750 mg/kg. There was a reduction in maternal body weight gain throughout the treatment period at 500 and 750 mg/kg. The reductions were statistically significant, except on GD 12–15 in the 500-mg/kg group. The corrected body weight gains were also significantly depressed in dams dosed with 500 and 750 mg/kg NMP (25% lower than control). Food consumption was less than that of control on GD 9–12 and 18–21 at 500 mg/kg, and during all the intervals measured at 750 mg/kg.

No significant effect of NMP was noted on the pregnancy rate and the number of corpora lutea and implantations sites (Table 2). There was a statistically significant increase in the rates of post-implantation loss and resorptions in the 500 mg/kg group relative to the concurrent control group. At 750 mg/kg, the number of live foetuses was greatly reduced due to a marked increase in the number of resorptions. Only eight out of 25 dams had live foetuses. The incidence of foetal deaths was low in all NMP-treated groups, but tended to increase with the dose. Doses of 250 to 750 mg/kg produced significant dose-related decreases in foetal body weights (males, females, total). These decreases amounted by 10, 30 and 47% at 250, 500 and 750 mg/kg, respectively. The percentage of male foetuses was higher in the 250 and 750 mg/kg NMP groups than in the controls. However, the lack of any effects at 500 mg/kg and in the dose range-finding studies suggest that this findingmay be a reflection of the variability seen in this strain.

The overall incidence of malformed foetuses per litter and the percentage of litters containing at least one malformed foetus were significantly increased at 500 and 750 mg/kg (Table 3). A number of external, visceral and skeletal malformations occurred only in NMP-treated groups, and a consistent dose-dependent trend was found in the incidence of these defects.

Although no significant difference was attained, NMP treatment was associated with an increased incidence of two types of external malformations: anasarca, and anal atresia associated with absent or vestigial tail. One or both were observed in one foetus after treatment with 250 mg/kg, in 11 foetuses from nine different litters after treatment with 500 mg/kg, and in one foetus after treatment with 750 mg/kg. Single instances of omphalocele, and of proboscis and cleft palate were also detected at 125 and 750 mg/kg, respectively.

Visceral examination revealed heart and/or great vessels malformations in 10 foetuses from nine litters in the 500-mg/kg group, and in six foetuses from four litters in the 750 mg/kg group. Their incidence was significantly increased at these two doses. Persistent truncus arteriosis was predominantly observed. Except for one control foetus showinganophthalmia, no other visceral malformation was seen.

There was a significant increase in the incidence of foetuses and litters with skeletal malformations at 500 and 750 mg/kg. No individual skeletal malformation was statistically different from control. The most prevalent malformations were fusion or absence of cervical arches. In addition to missingcaudal vertebrae, one foetus from the 500 mg/kg dose group showed no sacral centra, and another from a different litter exhibited

Table 1
Maternal parameters from Sprague–Dawley rats given NMP on GD 6–20 by gavage

	Dose (mg/kg/day)				
	0	125	250	500	750
No. treated	27	27	25	26	26
No. (%) pregnant at euthanization	21 (77.8)	22 (81.5)	24 (96.0)	25 (96.2)	25 (96.2)
No. of deaths	0	0	0	0	0
Body weight on day 0 (g)	232ff 12[a]	229ff 17	230ff 13	231ff 14	230ff 12
Body weight change (g)					
Days 0–6 (pretreatment period)	30ff 5	29ff 6	27ff 5	28ff 7	27ff 6
Days 6–9	12ff 6	12ff 4	9ff 4	8ff 4**	4ff 5 **
Days 9–12	16ff 5	16ff 4	14ff 4	13ff 4*	12ff 4 **
Days 12–15	19ff 6	20ff 4	21ff 5	16ff 4	6ff 9 **
Days 15–18	38ff 8	38ff 11	35ff 6	28ff 5 **	10ff 6 **
Days 18–21	49ff 15	48ff 12	42ff 9	38ff 9 **	6ff 10 **
Days 6–21 (treatment period)	134ff 30	134ff 28	122ff 18	102ff 15**	65ff 24**
Absolute weight gain[b]	38ff 12	40ff 11	34ff 8	28ff 13*	28ff 17*
Food consumption (g/day)					
Days 0–6	23ff 2	23ff 3	23ff 2	23ff 2	23ff 3
Days 6–9	24ff 3	24ff 3	23ff 2	22ff 1	22ff 3 *
Days 9–12	25ff 3	24ff 3	24ff 2	23ff 2*	22ff 2**
Days 12–15	26ff 3	26ff 2	25ff 2	24ff 2	23ff 4**
Days 15–18	28ff 3	28ff 3	27ff 2	26ff 2	24ff 3**
Days 18–21	27ff 4	27ff 2	25ff 2	23ff 3**	21ff 4**
Days 6–21	26ff 3	26ff 2	25ff 2	24ff 1**	22ff 3**

*,** Significant differences from the vehicle control $P < 0.05$ and $P < 0.01$, respectively.
[a] Values are expressed as meansff SD.
[b] Body weight gain during GD6–21 minus gravid uterine weight.

Table 2
Gestational parameters from pregnant Sprague–Dawley rats given NMP by gavage on GD 6–20

	Dose (mg/kg/day)				
	0	125	250	500	750
All litters[a]	21	22	24	25	25
No. of corpora lutea per dam	14.6ff 2.4[b]	14.6ff 1.6	14.3ff 1.9	14.5ff 1.7	14.8ff 1.7
Mean no. of implantation sites per litter	13.3ff 3.2	13.6ff 3.0	13.3ff 3.2	14.0ff 2.0	13.8ff 3.0
Mean% post-implantation loss per litter[c]	4.1ff 6.1	9.3ff 21.3	4.5ff 6.6	10.6ff 10.5*	94.2ff 11.2**
Mean% dead foetuses per litter	0.0ff 0.0	0.4ff 1.6	0.0ff 0.0	1.2ff 3.4	3.2ff 7.1
Mean% resorption sites per litter	4.1ff 6.1	8.9ff 21.2	4.5ff 6.6	9.4ff 8.9*	91.0ff 16.0**
Live litters[d]	21	21	24	25	8
Mean no. of live fetuses per litter	12.7ff 3.1	13.1ff 2.6	12.7ff 3.0	12.4ff 2.1	2.4ff 2.3**
Mean% male foetuses per litter	44.2ff 17.5	46.1ff 11.9	53.6ff 14.7*	50.4ff 17.5	91.7ff 17.8**
Foetal body weight (g)					
All foetuses	5.73ff 0.50	5.59ff 0.22	5.18ff 0.35**	4.02ff 0.21**	3.01ff 0.39**
Male foetuses	5.79ff 0.42	5.74ff 0.25	5.32ff 0.45**	4.18ff 0.22**	3.03ff 0.40
Female foetuses	5.62ff 0.50	5.47ff 0.20	5.02ff 0.29**	3.88ff 0.28**	3.09ff 0.47**

*, ** Significant differences from the vehicle control, $P < 0.05$ and $P < 0.01$, respectively.
[a] Includes all animals pregnant at euthanization.
[b] Values are expressed as means ff SD.
[c] Resorptions plus dead foetuses.
[d] Includes all animals with live foetuses at euthanization.

Table 3
Incidence of malformations in foetuses of Sprague–Dawley rats given NMP by gavage on GD 6–20

	Dose (mg/kg/day)				
	0	125	250	500	750
Total no. of foetuses (litters) examined[a]					
External	267 (21)	276 (21)	304 (24)	311 (25)	19 (8)
Visceral	134 (21)	138 (21)	152 (24)	156 (25)	10 (6)
Skeletal	133 (20)	138 (21)	152 (24)	155 (25)	9 (5)
External malformations[b]					
Anasarca	0	0	0	6 (5)	1 (1)
Proboscis	0	0	0	0	1 (1)
Cleft palate	0	0	0	0	1 (1)
Anal atresia and tail, absent or vestigial	0	0	1 (1)	7 (5)	0
Omphalocele	0	1 (1)	0	0	0
No. (%) of foetuses with external malformations	0	1 (0.4)	1 (0.3)	11 (3.5) **	3 (15.8)**
No. (%) of litters with external malformations	0	1 (4.8)	1 (4.8)	9 (36.0) **	3 (37.5) *
Mean % of foetuses with external malformations per litter	0	0.4ff 1.7[c]	0.3ff 1.7	3.3ff 5.0	20.8ff 36.5
Visceral malformations					
Anophthalmia	1 (1)	0	0	0	0
Cardiovascular malformations	0	0	0	10 # (9)	6 # (4)
Dextrocardia	0	0	0	1 (1)	0
Truncus arteriosus, persistent	0	0	0	5 (4)	2 (2)
Aorta, transposed	0	0	0	2 (2)	2 (2)
Aorta, overridingand/or enlarged and pulmonary artery, narrow	0	0	0	3 (3)	1 (1)
Interventicular septum defect, solitary	0	0	0	1 (1)	1 (1)
No. (%) of foetuses with visceral malformations	1 (0.7)	0	0	10 (6.4) *	6 (60.0)**
No. (%) of litters with visceral malformations	1 (4.8)	0	0	9 (36.0) *	4 (66.7) **
Mean % of foetuses with visceral malformations per litter	0.6ff 2.7	0	0	6.1ff 8.7	66.7ff 51.6 #
Skeletal malformations					
Facial bones, abnormal	0	0	0	0	1 (1)
Atlas and exoccipital, fused	0	0	0	1 (1)	2 (2)
Atlas, axis and/or cervical archs, fused	0	0	0	7 (5)	3 (2)
Cervical archs, absent[d]	0	0	0	2 (2)	1 (1)
Thoracic archs, fused	0	0	0	0	2 (2)
Thoracic centra second and/or fourth absent	0	0	0	2 (2)	0
Vertebrae, thoracic, lumbar, and/or sacral, absent	0	0	0	2 (2)	0
Sacral archs, fused	0	0	0	0	1 (1)
Ribs, absent	0	0	0	1 (1)	0
Ribs, fused	0	0	0	0	2 (2)
Cleft sternum	0	0	0	0	2 (2)
No. (%) foetuses with skeletal malformations	0	0	0	14 (9.0) **	5 (55.6)**
No. (%) litters with skeletal malformations	0	0	0	12 (48.0) **	3 (60.0) **
Mean % foetuses with skeletal malformations per litter	0	0	0	9.6ff 11.7 ##	46.7ff 44.7 ##
No. (%) foetuses with any malformations	1 (0.4)	1 (0.4)	1 (0.33)	30 (9.6) **	11 (57.9)**
No. (%) litters with any malformations	1 (4.8)	1 (4.8)	1 (4.2)	18 (72.0) **	6 (75.0) **
Mean % foetuses with any malformations per litter	0.3ff 1.4	0.4ff 1.7	0.3ff 1.7	9.6ff 8.3 ##	58.3ff 43.6 ##

[a] Only live foetuses were examined.
[b] The incidence of individual malformation is presented as number of foetuses (number of litters). A single foetus may be represented more than once in listingof the individual malformations
[c] Meanff SD.
[d] Absent = alizarine red S negative
* and ** significant differences from the vehicle control, $P < 0.05$ and $P < 0.01$, respectively, Fischer's test.
# and ## significant differences from the vehicle control, $P < 0.05$ and $P < 0.01$, respectively, Mann-Whitney test.

missingthoracic, lumbar and sacral vertebrae and missingribs. No external, visceral or skeletal malformation occurred at 125 mg/kg.

Several external and visceral variations were observed in single or few foetuses in the control and/or treated groups (Table 4). The percentage of foetuses with skeletal variations was significantly higher than controls at 500 and 750 mg/kg. This was largely due to increased incidences of poorly ossified skull bones (frontals, parietals and/or supraoccipital) and sternebrae. Although not significantly different, extra lumbar ribs, a common skeletal variant, was also observed more frequently.

Table 4
Incidence of variations in foetuses of Sprague–Dawley rats given NMP by gavage on GD 6–20

Dose (mg/kg/day)	0	125	250	500	750
Total no. of foetuses (litters) examined[a]					
External	267 (21)	276 (21)	304 (24)	311 (25)	19 (8)
Visceral	134 (21)	138 (21)	152 (24)	156 (25)	10 (6)
Skeletal	133 (20)	138 (21)	152 (24)	155 (25)	9 (5)
External variations[b]					
Nostril, misshapen	0	0	0	1 (1)	0
Club foot	0	1 (1)	1 (1)	1 (1)	0
No. (%) of foetuses with external variations	0	1 (0.4)	1 (0.3)	2 (0.6)	0
No. (%) of litters with external variations	0	1 (4.8)	1 (4.2)	2 (8.0)	0
Mean % of foetuses with external variations per litter	0	0.3ff 1.4[c]	0.3ff 1.3	0.5ff 1.9	0
Visceral variations					
Palate rugae, misshapen in the center of palate	0	0	0	1 (1)	0
Uterine horn, small and oviduct, misshapen	0	0	1 (1)	0	0
Ovaries, displaced	0	0	0	1 (1)	0
Testis, displaced	0	0	0	1 (1)	0
Kidney, small	0	0	0	0	1 (1)
Dilated renal pelvis	0	0	0	2 (2)	0
Distended ureter	4 (4)	0	1 (1)	1 (1)	2 (1)
No. (%) of foetuses with visceral variations	4 (3.0)	0	2 (1.3)	5 (3.2)	3 (30.0) **
No. (%) of litters with visceral variations	4 (19.0)	0	2 (8.3)	5 (20.0)	2 (33.3)
Mean % of foetuses with visceral variations per litter	2.7ff 5.8	0	1.3ff 4.4	3.3ff 7.0	16.7ff 27.9
Skeletal variations					
Skull, incomplete ossification[c]					
Frontals and parietal	1 (1)	0	0	55 ## (17)	8 ## (5)
Supraoccipital	1 (1)	0	0	13 (6)	8 ## (5)
Interparietal	1 (1)	0	0	0	0
Hyoid, absent[d]	1 (1)	0	0	0	0
Sternebrae					
First and second, fused	0	0	1 (1)	1 (1)	0
Incomplete ossification or absent					
No. 5 and/or 6	0	1 (1)	7 (7)	43 ## (21)	6 ## (5)
Other than no. 5 and/or 6	0	0	0	6 (5)	3 (3)
Rib(s)					
Cervical, rudimentary	2 (2)	1 (1)	6 (6)	19 (10)	1 (1)
14th, supernumerary	18 (8)	26 (13)	29 (13)	38 (18)	6 (3)
13th, short (uni or bilateral)	2 (1)	0	0	0	0
Thoracic vertebral centra					
First absent	0	0	0	2 (2)	2 (2)
Incomplete ossification (one or two)	13 (8)	7 (4)	3 (3)	15 (11)	5 # (4)
No. (%) of foetuses with skeletal variations	33 (24.8)	33 (23.9)	41 (27.0)	115 (74.2) **	9 (100.0) **
No. (%) of litters with skeletal variations	14 (70.0)	15 (71.4)	19 (79.2)	25 (100.0) *	5(100.0)
Mean % of foetuses with skeletal variations per litter	24.7ff 20.3	22.6ff 22.1	26.2ff 25.8	74.2ff 24.9 ##	100.0ff 0.0 ##

[a] Only live foetuses were examined.

[b] The incidence of individual defect is presented as number of foetuses (number of litters). A single foetus may be represented more than once in listingof the individual variations.

[c] Meanff SD.

[d] Absent = Alizarin Red S negative.

* and ** significant differences from the vehicle control, $P < 0.05$ and $P < 0.01$, respectively, Fischer's test.

and ## significant differences from the vehicle control, $P < 0.05$ and $P < 0.01$, respectively, Mann-Whitney test.

4. Discussion

Oral administration of 500 and 750 mg/kg to rats throughout the embryonic and foetal period resulted in obvious maternal toxicity, as evidenced by decreases in maternal weight gain over the treatment period. Absolute weight gain was also depressed. Hence, the body weight effects were not a consequence of reduced gravid uterine weights (i.e. a reflection of the reduced number of live foetuses and mean foetal weight). Maternal food consumption was also reduced duringthe later half of the dosing period at 500 mg/kg and throughout treatment at 750 mg/kg.

NMP caused dose-dependent adverse effects on the embryo/foetal development, includingembryolethality, teratogenicity and growth retardation.

The adverse effects of NMP on embryonic viability appeared at 500 mg/kg. Then, the dose-response for prenatal mortality appeared to be steep. Thus, the incidence of resorptions rose from 10% at 500 mg/kg to 91% at 750 mg/kg, and about two-thirds of the dams had complete resorbed litters at the high dose. These findings are sustained by the results of the pilot study in which rats showed 4, 18, 68 and 96% resorptions at 0, 500, 625 and 750 mg/kg, respectively.

The incidence of malformed foetuses and of litters with malformed foetus was significantly increased at 500 and 750 mg/kg. NMP elicited specific external, visceral and skeletal malformations, includinganasarca, anal atresia associated with absent or vestigial tail, cardiovascular malformations (mainly persistent truncus arteriosis), and various malformations of the spinal column (mainly fusion and/or absence of cervical arches). The spectrum of external malformations was consistent across the pilot and definitive studies. The individual malformations occurred at meaningful, but low incidence. Nevertheless, consideration must be given to the possibility that the teratogenic potential of NMP administered orally may have been masked by excessive embryolethality at the highest dose tested. At 250 mg/kg, a single foetus showed malformations similar to those recorded at higher doses, and they were considered related to treatment.

Foetal body weight was the most sensitive indicator of developmental toxicity, exhibitingsigificant reductions at doses 250 mg/kg or greater. Retarded development was also reflected by reduced ossification of skull bones and sternebrae at 500 and 750 mg/kg. No evidence of developmental toxicity was observed in the 125 mg/kg dose group.

Thus, the rat conceptus appeared to be more sensitive than the adult to the adverse effects of NMP when administered by gavage over the full period of embryo/foetal development. The LOAEL (lowest-observable-adverse-effect level) for maternal toxicity was 500 mg/kg and the NOAEL was 250 mg/kg. The developmental toxicity LOAEL was 250 mg/kg and the NOAEL was 125 mg/kg.

The results of this study employingoral administration contrast with previous inhalation studies in rats, in which no evidence of teratogenic effects was found. Exposure to 100 or 360 mg/m^3 NMP aerosols, for 6 h/day on days 6–15 of gestation did not affect pregnancy outcome or embryo/foetal development (Lee et al., 1987). Solomon et al. (1995) reported a slight decrease in foetal weight in the presence of marginal signs of maternal toxicity after exposure to 478 mg/m^3 NMP vapours, for 6 h/day, before and throughout gestation. Prenatal exposure to 680 mg/m^3 NMP for 6 h/day, from GD 4 through GD 20, resulted in increased preimplantation loss, lower body weights and delayed ossification in the absence of maternal toxicity (Hass et al., 1996). More severe deleterious effects on prenatal development have been observed followingdermal application of NMP to rats from GD 6–15 (Becci et al., 1982). Treatment with 750 mg/kg resulted in fewer live foetuses, and increased incidence of resorptions and skeletal defects (e.g. missing sternebrae, fused/split/extra ribs, fused atlas and occipital bones). The differences in the results might be due to route-related qualitative and quantitative differences in NMP bioavailability and/or metabolism. Only limited published information is available on NMP kinetics in experimental animals. Midgley et al. (1992) reported that NMP is readily and extensively absorbed followingdermal and oral administration in rats. NMP has been shown to reach the foetus after whole-body inhalation exposure of pregnant rats to 150 ppm NMP on GD 19 or 20 (Rav-Jonsen et al., 1992). Comparable maternal and foetal blood levels of NMP were found. Although further toxicokinetic studies usingvarious routes of administration are needed, it may be hypothesised that systemic exposure (maternal and/or embryofoetal) may be lower after inhalation exposure to NMP vapours than after ingestion of significant amounts of NMP.

Solomon et al. (1996) reported a human case of delayed foetal development followed by a stillbirth at week 31 of gestation. The mother worked in the electronics industry where she had used NMP and small amounts of acetone and methanol for the first 20 weeks of pregnancy. Levels of exposure to NMP (presumably via dermal contact and inhalation) were not exactly known. Although these authors claimed that it raised concern, they could not conclusively link the exposure to NMP with this single case of stillbirth (Bower, 1997; Solomon et al., 1997).

In summary, oral administration of NMP to rats duringthe embryonic and foetal periods can cause developmental toxic effects, includingembryolethality and teratogenicity, in the presence of maternal toxicity. Foetotoxicity, evidenced by decreased foetal body weight, occurred in the absence of maternal toxicity.

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